

Statistical inferences about the mechanism of action in carcinogenicity studies

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An innovative approach to dose–response modeling provides statistical insight into the relative likelihood of different mechanisms of action in cancer dose–response studies. Two illustrative examples are given based on time-to-tumor data on mammary fibroadenoma and adenocarcinoma in female Sprague-Dawley rats using 34 different dose metrics. The likelihood for the study outcome was calculated for each dose metric and compared with the background likelihood using a likelihood-ratio test. In the first example, fibroadenomas were strongly related to the presence or absence of mammary secretory activity, galactoceles, pituitary tumors, and abnormal diestrous days in weeks 1 to 26. Adenocarcinomas were the most strongly related to the number and percentage of abnormal estrous days. In these examples, the usual dose metric based on the dietary concentration of the pesticide had some explanatory ability but not nearly as much as the dose metrics more directly related to hormonal mechanisms of action.

Key terms cancer study; dose metric; dose–response modeling; likelihood ratio; maximum likelihood; mechanism of action; pesticide; statistical analysis.

In this paper the objective is to introduce and illustrate an innovative approach to dose–response modeling that provides statistical insight into the relative likelihood of different mechanisms of action in dose–response studies of cancer or other health effects.

Cancer animal bioassays or human epidemiologic studies are often used to help describe the shape of the dose–response relationship for a specific substance and extrapolate response characteristics from high to low doses. This paper shows how these data can also be used to provide an indication of the mechanism by which the substance causes the response.

The indication of the mechanism of action is obtained first by determining the maximum likelihood for the study outcome for each dose metric. The maximization is over the feasible values of the parameters in the specified dose–response model when the specified dose metric is used as the dose. Thereafter, the likelihood values corresponding to the different dose metrics are ranked. The mechanisms of action corresponding to the dose metrics with the highest likelihood, the second highest likelihood, and so forth are the mechanisms with the greatest probability of being associated with the response (greatest among the mechanisms being evaluat-

ed). This process ranks the mechanisms of action being evaluated and provides a quantitative indication of the relative strength of the support for the different mechanisms of action.

In this case, the terms “dose” and “dose metric” mean a measure of exposure (eg, parts per million or milligrams per kilogram per day) or a measure of the biological activity potentially generated by the exposure if a specific mechanism of action applies (eg, mammary secretory activity corresponding to a hormonal mechanism of action). The latter meaning of “dose” is consistent with the objective of having the dose term in the dose–response model be the characterization of the exposure or the observable consequence of the exposure that is as predictive of the response of concern as possible. This same motivation has, for example, led to the “administered dose” (concentration, etc) or the “internal dose” (intake, amount absorbed, etc) being replaced by the “delivered dose” (the amount of substance delivered to the target tissue). Furthermore, if something is known about what potentially relevant biological activity occurs as a result of the delivered dose, then a measure of that activity can replace the delivered dose as the dose term in the dose–response model.

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Statistical procedures are used to determine whether the likelihood using a specific dose metric is significantly greater than the "background" likelihood and whether the likelihood is significantly increased by incorporating additional mechanisms of action into the dose metric.

Methods

Dose-response studies are considered in which the response of interest is a quantal response such as the presence or absence of a tumor as opposed to a continuous response such as weight. The "study outcome" is defined as (i) the relevant data on each subject's exposure to a substance such as a pesticide (including any observable biological effects of the exposure that may be precursors to the specified response of primary interest), (ii) whether or not each subject had the specified response, and (iii) the time each subject was last observed for the presence or absence of the specified response.

In the examples herein, the subjects were assumed to be independent. The likelihood for the study outcome was defined as the product over all subjects ($i=1, 2, \dots, N$) of the i -th subject's probability of having (or not having) the specified response by (or at) the i -th subject's observation time. More specifically, let the i -th subject's dose and observation time be denoted by dose _{i} and t_i , respectively. Then the i -th subject's component in the product is

$$P_i = \text{Prob}(T \leq t_i | \text{dose}_i), \quad (\text{equation 1})$$

if all that is known about the time (T) to response for the i -th subject is that it was less than or equal to t_i (eg, response detected only at necropsy at time t_i). If the response occurred at time t_i , then the i -th subject's component in the product is

$$p_i = p(T = t_i | \text{dose}_i) = d/dt_i [P_i] = d/dt_i [\text{Prob}(T \leq t_i | \text{dose}_i)]. \quad (\text{equation 2})$$

Finally, if the i -th subject did not have the response by time t_i , then the i -th subject's component in the product is

$$1 - P_i = 1 - \text{Prob}(T \leq t_i | \text{dose}_i). \quad (\text{equation 3})$$

In the examples that follow, each subject's response probability, P_i , is modeled using a multistage-Weibull time-to-tumor model although any dose-response model could be used. The background likelihood is evaluated using the model in the form where

$$P_i = \text{Prob}(T \leq t_i | \text{Background}) = 1 - \exp[-b_0 \times t_i^{b_1}]. \quad (\text{equation 4})$$

In this form of the model, the time-to-response relationship is assumed to be the same for all the subjects

regardless of their exposure. The background likelihood is defined to be the maximum of the study outcome's likelihood where the maximum is determined over all feasible values of parameters b_0 and b_1 .

The likelihood corresponding to any specified dose metric, D , is the likelihood with

$$P_i = \text{Prob}(T \leq t_i | D_i) = 1 - \exp[-(\delta_0 + \delta_1 \times D_i) \times t_i^{\delta_2}], \quad (\text{equation 5})$$

after it has been maximized over all feasible values of the parameters δ_0 , δ_1 , and δ_2 .

The likelihood can be calculated for several potentially relevant dose metrics. The greater the value of the likelihood, the more likely that the study outcome is "explained" by that dose metric. The dose metric with the greatest likelihood corresponds to the most likely mechanism of action (or, more precisely, "most likely" among the mechanisms corresponding to the dose metrics evaluated and for the dose-response model used). The ratio of the likelihoods for two different dose metrics indicates the relative likelihoods of the two corresponding mechanisms of action.

A dose metric corresponds to a statistically significant increase in the likelihood (at an $\alpha \times 100\%$ significance level) compared with the background likelihood if the natural logarithm of the ratio of the likelihood corresponding to a specific dose metric to the background likelihood is greater than 0.5 times the $100 \times (1 - \alpha)$ -th percentile of a chi-square distribution with one degree of freedom. That is, if

$$\ln(\text{likelihood using dose metric} / \text{background likelihood}) > 0.5 \times \chi^2_{1, 1-\alpha}. \quad (\text{equation 6})$$

Results

In the first example, the response in the 104-week study was the presence of a palpable mammary fibroadenoma in a female Sprague-Dawley rat exposed to a pesticide in its diet. Figure 1 shows the ratio of the likelihood corresponding to each of 34 candidate dose metrics to the background likelihood, that is, the fold increase in the likelihood when each candidate dose metric is used to try to explain the study outcome.

In figure 1, dose metric number 1 is the concentration (parts per million) of a pesticide in the diet. This dose metric increases the likelihood about threefold when compared with the background likelihood. This increase is not statistically significant at the 5% significance level. Thus, while dietary concentration has some explanatory ability, it is not much better than assuming that the time-to-response relationship is the same for all subjects regardless of their exposure. On the other hand, the dose metrics in which dietary concentration is replaced by the number or percentage of days that the

subject is in diestrus, estrus, or abnormal estrus during weeks 1 to 50 of the study do significantly increase the likelihood of the study outcome with respect to fibroadenomas. Furthermore, when the dose metric corresponds to the presence (1) or absence (0) of mammary secretory activity, the likelihood is greatly increased. Thus, in this first example, the mechanism of action for fibroadenomas in female Sprague-Dawley rats is the most consistent with a hormonal mechanism related to mammary secretory activity and, to a less extent, to disruptions of the estrus cycle. Here, the pesticide exposure affects the frequency of fibroadenomas, and the most likely mechanism of action is hormonal.

Figure 2 refers to the dose-response relationship for carcinomas (adenocarcinomas as opposed to fibroadenomas) in the same study. For carcinomas, the likelihood is significantly increased over the background likelihood when the dose metric is dietary concentration (ppm) but is increased even more using some of the other dose metrics. Hence, figure 2 shows the fold increase in the likelihood for different dose metrics compared with the likelihood using parts per million (dose metric 1) as opposed to the background likelihood. (Trivially, the fold increase for dose metric 1 is 1.0 because the likelihood using dose metric 1 divided by itself is 1.0.) For carcinomas, the greatest likelihoods and hence the most likely mechanisms of action are hormonal mechanisms primarily related the number or percentage of abnormal days in estrus.

Thus the most likely mechanism of action in both cases is hormonal, but it is the most closely linked to the mammary secretory activity for fibroadenomas and to abnormal estrous days for carcinomas.

Figure 3 shows the fold increase in the likelihood for fibroadenomas when the dose metric is expanded from including just the presence or absence of mammary secretory activity to also including other explanatory variables. The likelihood is increased the most when the presence or absence of mammary galactoceles is added to the dose metric. In this case, the dose-response model is expanded from

$$P_i = P(T \leq t_i | MS) = 1 - \exp [-(\delta_0 + \delta_1 \times MS) \times t_i^{\delta_2}], \quad (\text{equation 7})$$

where MS equals 1 if there is mammary secretory activity and equals 0 otherwise, to

$$P_i = P(T \leq t_i | MS \text{ and } MG) = 1 - \exp [-(\lambda_0 + \lambda_1 \times MS + \lambda_2 \times MG) \times t_i^{\lambda_3}], \quad (\text{equation 8})$$

where MG equals 1 if there are mammary galactoceles and equals 0 otherwise.

Follow-up analyses to those shown in figure 3 showed that the likelihood for fibroadenomas was statistically significantly increased still further if the dose

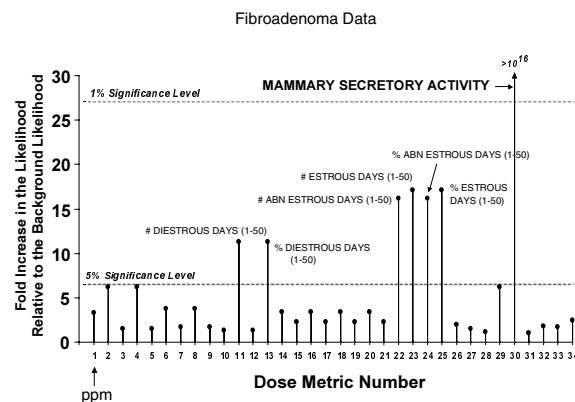


Figure 1. Fold increase in the likelihood for fibroadenoma incidence and the time to occurrence for different dose metrics relative to the background likelihood. (ABN = abnormal, ppm = parts per million)

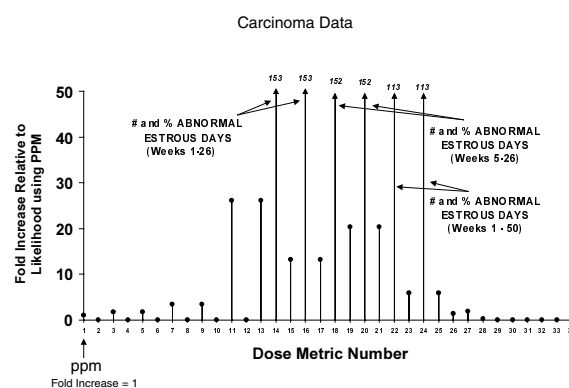


Figure 2. Fold increase in the likelihood for carcinoma incidence and the time to occurrence for different dose metrics relative to the likelihood, using dietary concentration as the dose metric. (PPM or ppm = parts per million)

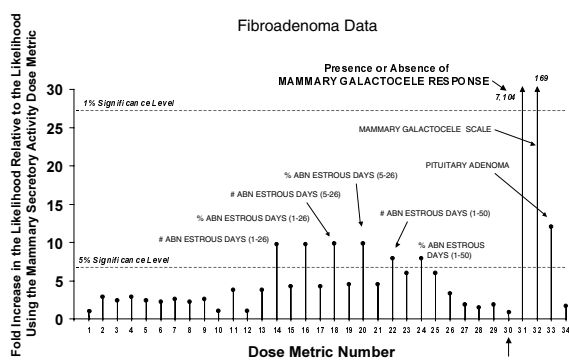


Figure 3. Fold increase in the likelihood for fibroadenomas when the dose metric for mammary secretory activity is supplemented with different dose metrics relative to the likelihood, using presence or absence of mammary secretory activity as the dose metric.

metric was further expanded to include the presence or absence of pituitary adenomas and then expanded to also include the number or percentage of abnormal diestrus days in weeks 1 to 26. No further expansions resulted in statistically significant increases in the likelihood.

Discussion

In the fibroadenoma example, there was a tendency for the response frequency to increase with pesticide exposure when the dose metric was simply the dietary concentration (parts per million) of the pesticide (figure 4). However, the dose-response relationship is not clear. That is, fibroadenomas may be related to the dietary

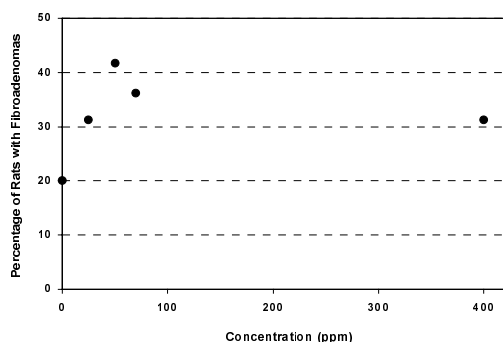


Figure 4. Percentage of female Sprague-Dawley rats with fibroadenomas in a treatment or control group versus the pesticide concentration in the diet. (ppm = parts per million)

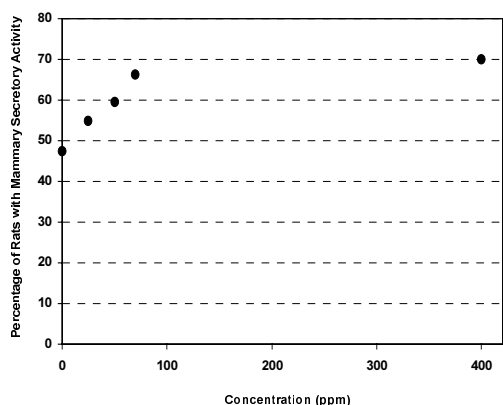


Figure 5. Percentage of female Sprague-Dawley rats with mammary secretory activity in a treatment or control group versus the pesticide concentration in the diet. (ppm = parts per million)

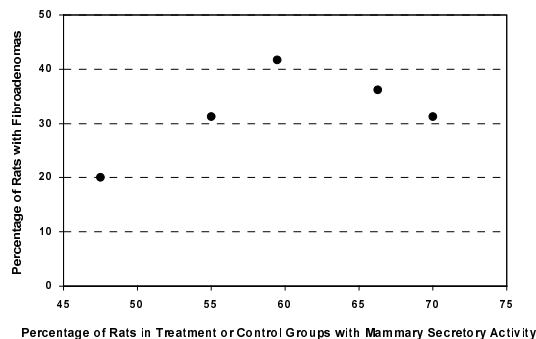


Figure 6. Percentage of female Sprague-Dawley rats with fibroadenoma in a treatment or control group versus the percentage of rats with mammary secretory activity in the group.

concentration of the pesticide, but it is not obvious how or why.

Figure 5 shows that the presence or absence of mammary secretory activity in subjects is related to pesticide exposure.

If the dietary concentration is replaced in the dose-response model by the percentage of animals with mammary secretory activity in each treatment group (including controls), then the dose-response relationship is a little clearer (figure 6). There is a suggestion that the effect of the pesticide exposure on fibroadenoma frequency occurs through the mammary secretory activity associated with that exposure.

The study data include the time that the female Sprague-Dawley rat was checked for a palpable fibroadenoma. Hence there is more information about the responses in the control and treatment groups than is indicated in figures 5 and 6. The time-to-response data for all the groups combined can be fit using a multistage-Weibull model with the dose metric being the dietary pesticide concentration. The data can also be fit with the dose metric being the presence or absence of mammary secretory activity. As shown in figures 7 and 8, the fit with the mammary secretory dose metric is much better than the fit with the dose metric being the dietary pesticide concentration. Figure 1 indicates how much better, in the sense that the part-per-million dose metric increases the likelihood about threefold above the background likelihood, whereas the mammary secretory dose metric increases the likelihood more than 10^{16} times above the background likelihood.

Just as the correlation (r) or the coefficient of determination (r^2) are used to describe the strength of simple linear relationships, the likelihood and likelihood ratios can be used to describe and compare the explanatory power of alternative dose metrics. The mechanisms of

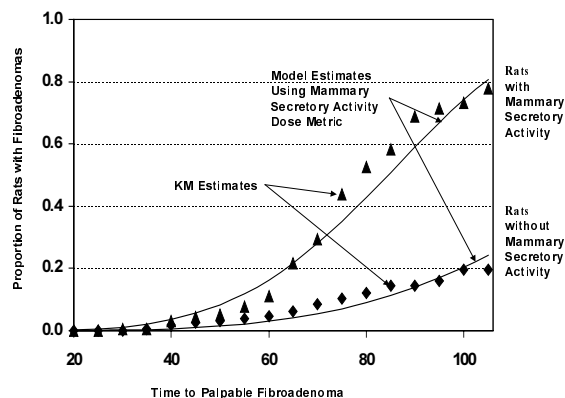


Figure 7. Multistage-Weibull model and nonparametric Kaplan-Meier (KM) estimates of the cumulative distribution function for the time to palpable fibroadenoma in female Sprague-Dawley rats with and without mammary secretory activity when the dose metric in the multistage-Weibull model is the presence or absence of mammary secretory activity.

action associated with the dose metrics with the greatest likelihoods are the mechanisms of action that are the most likely to be causing the specified response.

Although the functions of dose in the multistage-Weibull time-to-response model have frequently been simple linear functions of a single dose metric, these functions could be functions of multiple dose metrics and be nonlinear. The situations and issues are analogous to those associated with regression analyses. Here, the regression analyses are imbedded within a dose-response model.

Concluding remarks

Statistical analyses of data from cancer and noncancer studies often focus on the dose-response relationship. These analyses can help quantify how the response frequency changes with the dose level. In addition, the use of different dose metrics and the likelihood-based techniques described herein can help identify how the pesticide or other substance causes the response and the relative likelihood of different mechanisms of action.

It is possible for a pesticide to have multiple modes of action and that tumor findings may be independent of other pesticide effects. However, in the examples herein, the likelihood-based statistical analyses strongly suggest that the hormonal mechanism of the pesticide action in female Sprague-Dawley rat altered estrus and in turn tumorigenesis. This finding is consistent with the biology since it is well known that alterations of estrus in this species result in changes in tumor responses.

The approach used to determine the relative likelihood of different mechanisms of action for a single substance can be generalized to a situation in which exposure may involve multiple substances. In this case, the different dose metrics could correspond to different

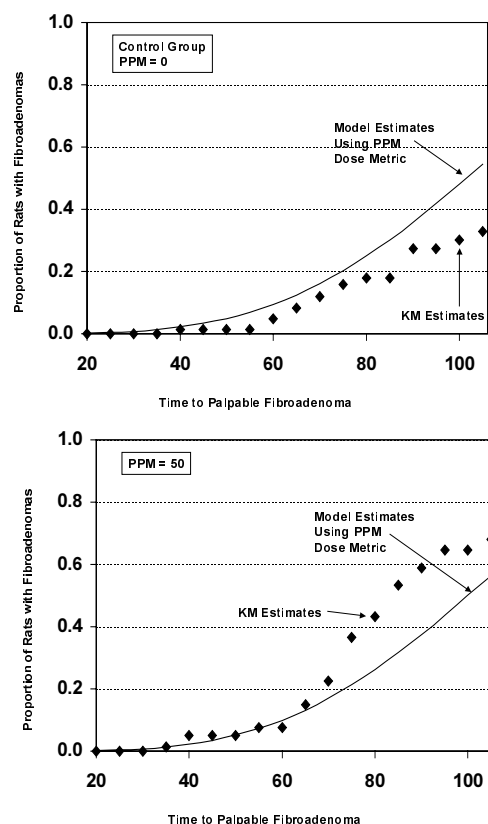


Figure 8. Multistage-Weibull model and nonparametric Kaplan-Meier (KM) estimates of the cumulative distribution function for the time to palpable fibroadenoma in female Sprague-Dawley rats with and without mammary secretory activity when the dose metric in the multistage-Weibull model is dietary concentration. (PPM = parts per million)

substances, as well as different mechanisms of action. The objective would be to determine the relative likelihood that different substances are associated with the specified response of interest and the potential mechanisms of action of these substances.